The Role of a Dopamine Stimulated G_q Pathway in the Development of Striatal Medium Spiny Neurons

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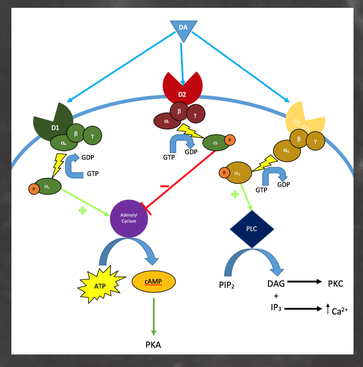
Abstract

There exists considerable evidence linking the role of dopamine receptor activation of Medium Spiny Neurons (MSNs) in the striatum to neurite development. A better understanding of this link between receptor activation and dendrite growth and plasticity can significantly contribute towards the understanding of ailments including Huntington's disease, depression, and addiction. MSNs cultured in the presence of dopamine (+DA) show significant dendrite growth and arborization compared to controls. Interestingly, cultures treated with dopamine stimulatory (D1) and dopamine inhibitor (D2) specific receptor agonists show little to no change in dendrite development compared to -DA controls. These results implicate an alternative dopamine mechanism separate from that of the traditional D1 and D2 pathways. Previous work has shown that the use of a G_q coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD), selectively activated by clozapine-n-oxide (CNO), exhibits dendrite growth similar to that observed in +DA MSNs. In our current study, we tested the potential role of a G_q coupled dopamine receptor in MSN development using the D1-D2 selective agonist SKF83959.

Background

- The striatum is a subnucleus of the basal ganglia with the primary cell population consisting of Medium Spiny Neurons (MSNs)[4]
- Abnormal striatal functioning has been linked to ailments including Parkinson's disease, Huntington's disease, and addiction[1,2,8]
- MSNs receive both dopamine and glutamate innervation from the cortex and midbrain respectively[6,7]
- The prevailing model of MSNs shows them as expressing either D1 or D2 dopamine receptors[5]
  - D1 pathway – G_s ; Activates adenylyl cyclase
  - D2 pathway – G_i ; Blocks adenylyl cyclase



Hypothesis

- If MSN treatment with a dopamine D1 and D2 receptor agonists fails to replicate the dendrite growth observed when cells are cultured with dopamine, then dopamine is likely acting through a different mechanism
  - The activation of a transfected G_q pathway will mimic results of cells cultured in the presence of dopamine
  - MSNs treated with the D1-D2 receptor agonist SKF83959 will mirror results gained from transfected G_q treatment and cells cultured with dopamine

Methods

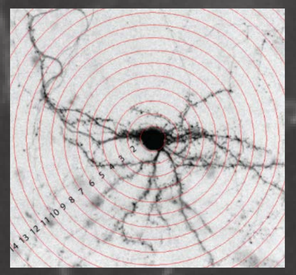
The striatum and cortex were removed from E15-16 mice and combined at a 3 cortex : 2 striatum ratio in culture

Selected culture suspensions were transfected with G_q DREADD via electroporation

Cell cultures were treated under the following conditions:
- DA-
- DA+
- SKF83959
- G_q DREADD +CNO
- G_q DREADD +DA

After 19 days, cultures were fixed, permeabilized, and immunostained for DARPP32, DAPI and G_q DREADD receptors

DARPP32 positive neurons were imaged using a Zeiss Axiovert M200 fluorescent microscope and Micro-Manager software



Imaged MSNs were traced using a ImageJ (NIH) FIJI plugin and analyzed with a Sholl analysis

Conclusions

- Our results indicate that there is likely a dopamine and G_q receptor mediated mechanism that is responsible for the arborization of MSNs in development
  - Dopamine present in cultures increases arborization between 13 and 19 div
  - The presence of D1 and D2 receptor agonists fails to mimic the increased distal arborization seen in the presence of dopamine
  - Inhibition of PLC blocked the developmental effects observed with the presence of dopamine
  - MSNs transfected with a designer G_q receptor showed developmental effects similar to that of dopamine treated cells

Future Directions:
- Test MSN cell cultures on the possible role of a D1-D2 receptor heter0dimer
  - D1-D2 homodimers work through a G_q-dopamine mediated mechanism, similar to the results we've obtained
  - The drug SKF83959 has shown D1-D2 receptor specificity at concentrations lower than 100uM
- Test whether the developmental affects seen in cultures treated with dopamine are directly affecting MSNs or indirectly acting through cortical neurons

Results

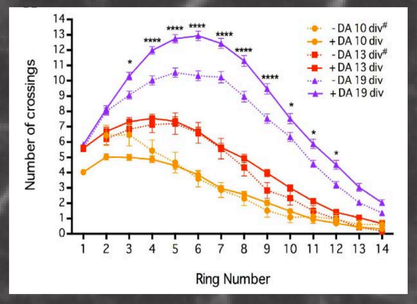


Figure 1: Most dendrite arborization occurred between 13-19 div and peaked around ring number 5 and 6 (50-60um from the soma). For 19 div cultures treated with dopamine (+DA), there was a significant increase in ring crossings, signifying increased arborization[3].

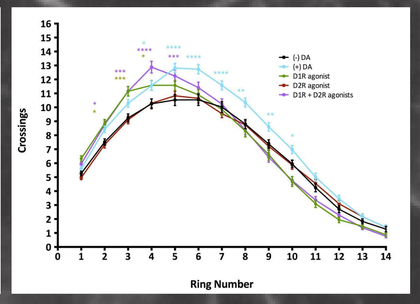


Figure 2: MSNs cultured with D1 receptor agonist (SKF) and/or D2 receptor agonist (quinprole) failed to replicate the increase in arborization observed in cultures treated with dopamine[3]. Suggesting that dopamine is acting via a non-traditional pathway.

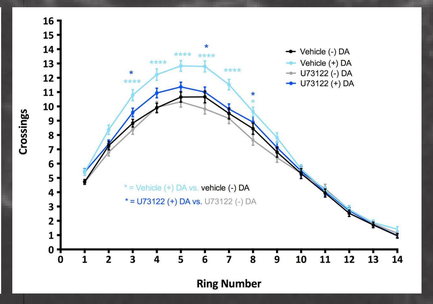


Figure 3: The effects of dopamine on dendritic arborization in MSN cell cultures was significantly decreased in the presence of the phospholipase-C antagonist U73122[3]. This suggests the role of a G_q pathway.

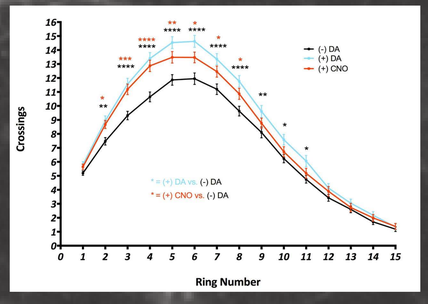
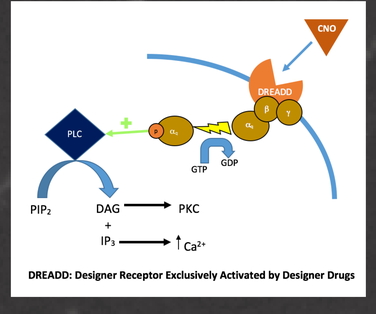


Figure 4: When MSNs were transfected with a designer G_q receptor specifically activated by CNO (DREADDs), there was a significant increase in dendrite arborization that was comparable to cells that received dopamine treatment. These cells also had significantly greater arborization than cells not exposed to dopamine[9].



DREADD: Designer Receptor Exclusively Activated by Designer Drugs

Ackowledgements and References

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